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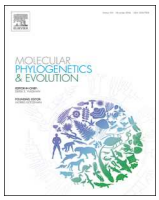

An alpine grasshopper radiation older than the mountains, on Kā Tiritiri o te Moana (Southern Alps) of Aotearoa (New Zealand)

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ABSTRACT

In New Zealand, 13 flightless species of endemic grasshopper are associated with alpine habitats and freeze tolerance. We examined the phylogenetic relationships of the New Zealand species and a subset of Australian alpine grasshoppers using DNA sequences from the entire mitochondrial genome, nuclear 45S rRNA and Histone H3 and H4 loci. Within our sampling, the New Zealand alpine taxa are monophyletic and sister to a pair of alpine Tasmanian grasshoppers. We used six Orthopteran fossils to calibrate a molecular clock analysis to infer that the most recent common ancestor of New Zealand and Tasmanian grasshoppers existed about 20 million years ago, before alpine habitat was available in New Zealand. We inferred a radiation of New Zealand grasshoppers ~13–15 Mya, suggesting alpine species diversification occurred in New Zealand well before the Southern Alps were formed by the mountain building events of the Kaikoura Orogeny 2–5 Mya. This would suggest that either the ancestors of today's New Zealand grasshoppers were not dependent on living in the alpine zone, or they diversified outside of New Zealand.

1. Introduction

Just as oceanic islands are influential in partitioning populations and facilitating evolution of distinctive traits (Darwin, 1859; Gillespie and Baldwin, 2009), other patchily distributed habitats with abrupt environmental boundaries or steep gradients harbour distinct ecological assemblages (DeChaine and Martin, 2005; Hughes and Eastwood, 2006; Knowles, 2001). A striking instance is the occupation of alpine environments by groups of related species specialised to the local conditions. This endemicity, which is particularly well observed in flowering plants, provides some of the most compelling examples of species radiation, where adaptive diversification is coupled with ecological release (Hughes and Atchison, 2015). Many of the alpine animal species radiations that have been explored are insects (e.g. *Melanoplus* grasshoppers (Knowles and Otte, 2000); *Maoricicada* cicadas (Buckley and Simon, 2007), *Nebria* ground beetles (Slatyer and Schoville, 2016)). In most instances these species radiations have been interpreted as recent and thus rapid (Linder, 2008), and linked to the relatively abrupt generation of novel habitats via regional orogenics.

Alpine habitat is characterised by low growing herbs and grasses above an elevational treeline (Prentice et al., 1992). In New Zealand (Aotearoa), the Southern Alps (Kā Tiritiri o te Moana) extend the length of the South Island (Te Wai Pounamu), perpendicular to the prevailing westerly air flow across the Tasman Sea (Te Tai-o-Rēhua). This yields an intense orographic precipitation gradient from west to east. When humans arrived ~650 years ago, New Zealand was dominated by forest (Argiriadis et al., 2018; McGlone, 1989), with open habitat limited to mountain peaks and semi-arid lowland areas immediately east of the Southern Alps (Alloway et al., 2007; Ausseil et al., 2011; McGlone, 1989) (Fig. 1). The existing high elevation alpine and low elevation semi-arid habitats both resulted from the emergence of the Southern Alps.

Tectonic activity along the New Zealand Alpine Fault at the contact between the Australian and Pacific continental plates started ~25 Mya, and involved > 700 km of lateral displacement (~30 mm/year) (Lamb et al., 2016). This Miocene tectonic activity led to the formation of the majority of New Zealand's land surface from the submerged continent Zealandia (Mortimer and Campbell, 2014; Trewick et al., 2007). However, the Southern Alps formed about 5 Mya with an accelerated rate of uplift (10–20 mm/year) along the plate boundary (Kamp, 1986; Trewick and Bland, 2012). An increase in sedimentation rates on the nearby continental shelf during the early Pliocene (6 Mya) is consistent with increased elevation (Lu et al., 2005). This likely yielded new ecological opportunities for diversification (Steinbauer et al., 2016) including the first alpine habitat in the Zealandia/New Zealand region since the Jurassic/Cretaceous (Batt et al., 2004; Chamberlain and Poage, 2000; Cockayne, 1921; Tippett and Kamp, 1993; Wallis and Trewick, 2009).

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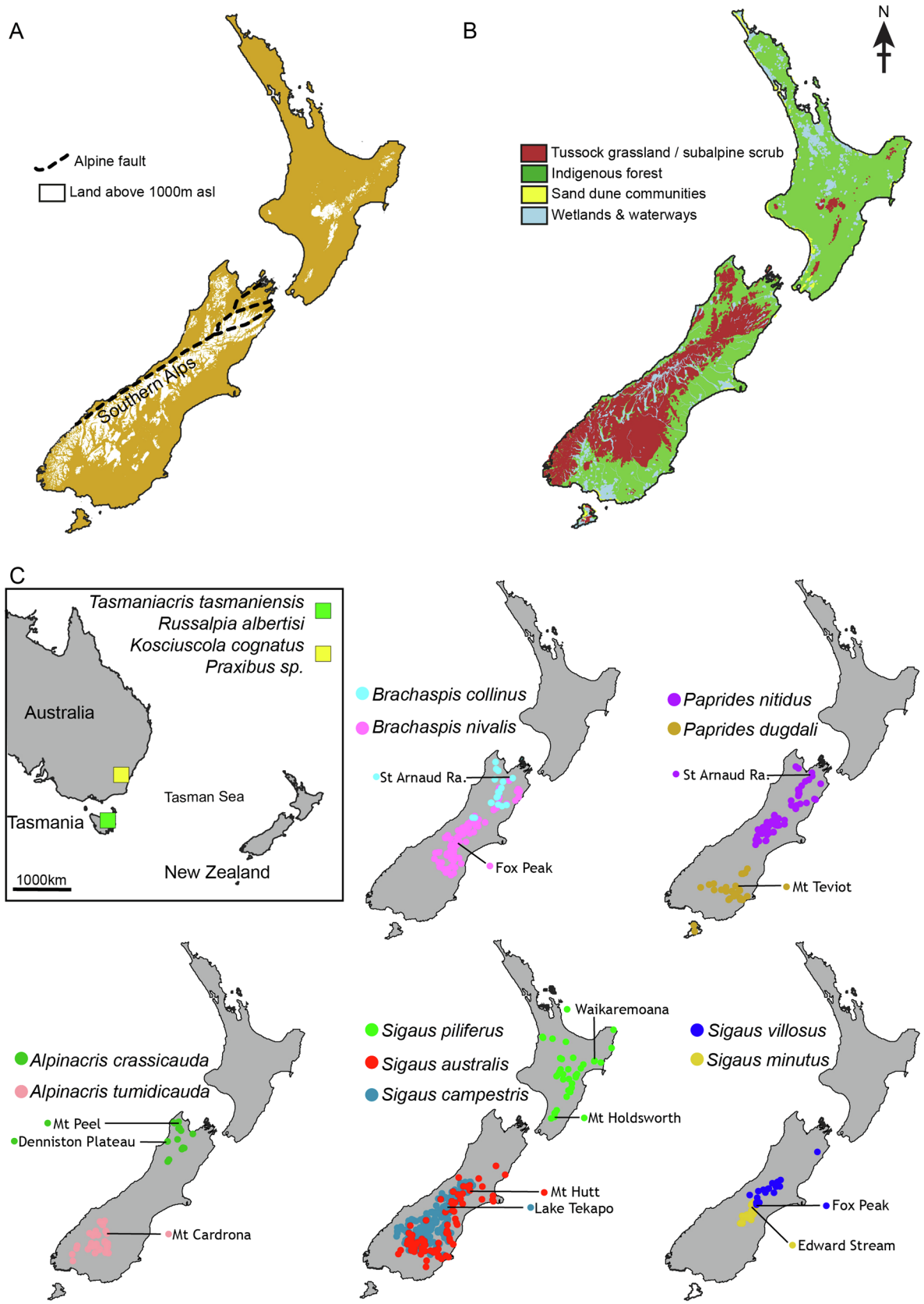

Fig. 1. The alpine landscape of New Zealand. (A) Montane regions (white above 1000 m asl) after Trewick and Bland (2012). (B) Distribution of alpine associated tussock grassland and other vegetation prior to the arrival of people in New Zealand (after Sivyer et al. 2018). (C) Sampling sites of New Zealand and Australian taxa used for whole mtDNA, nuclear 45S rRNA, and Histone H3 and H4 sequencing and recorded range of eleven New Zealand alpine grasshopper species.

Table 1
Sampling details of Orthoptera: Caelifera & Ensifera used in phylogenetic analysis of the New Zealand short-horn grasshopper radiation. > 21,000 bp of DNA sequence data comprised 45S rRNA cassette (~3800 bp), histones H3 and H4 (723 bp) and entire mtDNA genomes (15,600 bp). Taxa newly sequenced in this study are in bold. NZ = New Zealand. Locations in capitals are approximate regions where details were not provided. Specimen codes are linked with specimens kept in the Phoenix Collection at Massey University, Palmerston North. Details of taxonomic classification, global range and GenBank accession numbers are shown.

Classification	Species	Location	45S rRNA GenBank	Histone GenBank	Mtdna GenBank	Source
Acridoidea, Acrididae, Acridinae	*Phlaeoba albonema*	Tianhetan, Guiyang, China			NC011827	Shi et al. 2008 DS
	Phlaeoba tenebrosa	South and East Asia			NC029150	Song et al. 2016
Calliptaminae	*Calliptamus abbreviatus*	China			NC030626	Han et al. 2016 DS
	Calliptamus italicus	Brje pri Komnu, Slovenia			NC011305	Fenn et al. 2008
	Peripolus nepalensis	China			NC029135	Zhi et al. 2016 DS
Caryandinae	*Caryanda* sp.	China			NC030165	Mao and Hu 2016
Catantopinae	***Alpinacris crassicauda***	**Mt Peel, Tasman Mnts, NZ. AC25**	**MT072989**	**MT070974**	**MN253105**	**This study**
	***Alpinacris crassicauda* DP**	**Denniston Plateau, Westport, NZ. GH749**	**MT072988**	**MT070973**	**MN253103**	**This study**
	Alpinacris tumidicauda	**Mt Cardrona, NZ. GH1295**	**MT072994**	**MT070975**	**MN253104**	**This study**
	Brachaspis collinus	**St Arnaud Range, NZ. GH1405**	**MT072990**	**MT070976**	**MN253106**	**This study**
	***Brachaspis nivalis* SA**	**St Arnaud Range, NZ. GH1404**	**MT072991**	**MT070978**	**MN253108**	**This study**
	***Brachaspis nivalis* FP**	**Fox Peak, NZ. GH1371**	**MT072992**	**MT070979**	**MN253107**	**This study**
	Paprides dugdali	**Mt Teviot, NZ. GH830**	**MT072993**	**MT070980**	**MN253110**	**This study**
	Paprides nitidus	**St Arnaud Range, NZ. GH1407**	**MT072985**	**MT070981**	**MN253111**	**This study**
	Phaulacridium marginale	**Ahimanawa Range, NZ. GH762**	**MT072982**	**MT070992**	**MN253112**	**This study**
	Phaulacridium otagoense	**Lake Dunstan, NZ. GH1576**	**MT072983**	**MT070989**	**MN253113**	**This study**
	Sigaus australis	**Mt Hutt, NZ. GH1387**	**MT072995**	**MT070983**	**MN253117**	**This study**
	Sigaus campestris	**Lake Tekapo, NZ. GH1036**	**MT072996**	**MT070984**	**MN253118**	**This study**
	Sigaus minutus	**Edward Stream, Tekapo, NZ. GH433**	**MT072984**	**MT070985**	**MN253116**	**This study**
	***Sigaus piliferus* LW**	**Lake Waikaremoana, NZ. GH5**	**MT072986**	**MT070986**	**MN253120**	**This study**
	***Sigaus piliferus* MH**	**Mount Holdsworth, NZ. GH60**	**MT072987**	**MT070987**	**MN253119**	**This study**
	Sigaus villosus	**Fox Peak, NZ. GH1374**	**MT072997**	**MT070977**	**MN253121**	**This study**
	Russalpia albertisi	**Mt Wellington, Tasmania. TAZ3**	**MT072999**	**MT070982**	**MN253115**	**This study**
	Tasmaniacris tasmaniensis	**Mt Wellington, Tasmania. TAZ4**	**MT072998**	**MT070988**	**MN253122**	**This study**
	Traulia szetschuanensis	East Asia			NC013826	Huang and Zhang 2008a DS
	Xenocatantops brachycerus	China			NC021609	Liu and Huang 2013a DS
Cyrtacanthacridinae	*Chondracris rosea*	China			NC019993	Jiang and Qiang 2009a. DS
	Schistocerca gregaria	South Africa			NC013240	Erler et al. 2010
Eyprepocnemidinae	*Shirakiacris shirakii*	East and Southeast Asia			NC021610	Liu and Huang 2013b DS
Gomphocerinae	*Arcyptera coreana*	Shaanxi Province, China			NC013805	Huang and Liu 2009 DS
	Ceracris kiangsu	Purple Mountain, Nanjing, China			NC019994	Jiang et al. 2009b DS
	Ceracris versicolor	China			NC025285	Xu et al. 2016
	Chorthippus chinensis	Jiugongshan, Hubei, China			NC011095	Liu and Huang 2007 DS
	Euchorthippus fusigeniculatus	Helongjiang, China			NC014449	Zhao et al. 2010
	Gomphocerippus rufus	China			NC014349	Sun et al. 2010
	Gomphocerus licenti	China			NC013847	Gao and Huang 2009 DS
	Gomphocerus sibiricus	Central and West Asia, Europe			NC021103	Zhang et al. 2013
	Gomphocerus sibiricus	Central Asia			NC015478	Yin et al. 2012
	Gonista bicolor	East and Southeast Asia			NC029205	Zhang et al. 2016a
	Pacris xizangensis	South Asia			NC023919	Zhang et al. 2016b
Melanoplinae	*Fruhstorferiola kulinga*	East Asia			NC026716	Yang et al. 2016
	Fruhstorferiola sp.	East asia			KU355786	Guan and Xu 2015b DS
	Kingdonella bicollina	Nangqian, Qinghai, China			NC023920	Zhi et al. 2016
	Ognevia longipennis	Central and East Asia			NC013701	Huang and Zhang 2008b DS
	Prumna arctica	Mohe, Heilongjiang, China			NC013835	Sun et al. 2010
	Qinlingacris elaeodes	Taibai Mnts, Shaanxi, China			KM363599	Li et al. 2016
	Qinlingacris taibaiensis	East Asia			NC027187	Guan and Xu 2015a DS
Oxyinae	***Kosciuscola cognatus***	**Island Bend, Kosciuscola NP, Australia GH1721**	**MT073001**	**MT070990**	**MN253109**	**This study**
	Oxya chinensis	Chang'an, Xi'an, Shaanxi, China			NC010219	Hang and Zhang 2007 DS
	Oxya hyla inricata	East and Southeast Asia			KP313875	Dong et al. 2016
	Praxibulus sp.	**Island Bend, Kosciuscola NP, Australia GH1722**	**MT073000**	**MT070991**	**MN253114**	**This study**
Spathosterninae	*Spathosternum prasiniferum*	Yaoshan, Guangxi, China			KM588074	Zhou and Huang 2016

(*continued on next page*)

Table 1 (*continued*)

Classification	Species	Location	45S rRNA GenBank	Histone GenBank	Mtdna GenBank	Source
Pamphagoidea, Pamphagidae, Thrinchinae	*Asiotmethis jubatus*	Qinghe, Xinjiang Uygur, China			NC025904	Li et al. 2015
	Filchnerella helanshanensis	Inner Mongolia, China			NC020329	Zhang et al. 2013
	Prionotropis hystrix	South Europe			JX913764	Leavitt et al. 2013
Eumasticoidea Chorotypidae Erianthinae	*Erianthus versicolor*	Southeast Asia			JQ975394	Wei and Huang 2012 DS
Episactidae Episactinae	*Pielomastax zhengi*	East Asia			NC016182	Yang and Huang 2011
Eumastacidae Paramastacinae	*Paramastax nigra*	Western South America			JX913772	Leavitt et al. 2013
Tetrigoidea Tetrigidae Tetriginae	*Alulatettix yunnanensis*	East Asia			NC018542	Xiao et al. 2012a
	Tetrix japonica	Nanjing, Jiangsu, China			NC018543	Xiao et al. 2012b
Tridactyloidea Cylindrachetidae	*Cylindraustralia* sp.	Australia			KM657334	Song et al. 2015
Ripipterygidae Ripipteryginae	*Mirhipipteryx andensis*	Central and South America			NC028065	Song et al. 2015
Tridactylidae Tridactylinae Ensifera	*Ellipes minuta*	North, Central and South America			NC014488	Sheffield et al. 2010
Schizodactyloidea Schizodactylidae Comicinae	*Comicus campestris*	Southern Africa			NC028062	Song et al. 2015
Gryllidea Gryllotalpoidea Gryllotalpinae	*Gryllotalpa orientalis*	Suwon City, Gyunggi-do, Republic of Korea			AY660929	Kim et al. 2005
Grylloidea Gryllidae Gryllinae	*Teleogryllus emma*	Cosmopolitan			EU557269	Ye et al. 2008 DS

DS- Direct submission to GenBank.

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Associated with this open, alpine habitat in New Zealand are an array of animal species radiations including geckos (Nielsen et al., 2011), cicada (Buckley and Simon, 2007; Marshall et al., 2008), cockroaches (Chinn and Gemmell, 2004), carabid beetles (Goldberg et al., 2014), and stoneflies (McCulloch et al., 2010). There is a rich specialised flora (Raven, 1973), with several endemic radiations that evolved following recent long distance dispersal (Lockhart et al., 2001; Winkworth et al., 2005). Other lineages such as *Phyllache* (Wagstaff and Wege, 2002) and *Gaultheria* (Bush et al., 2009) appear to have established in New Zealand before alpine habitat was formed. The processes underlying the formation of this endemic alpine biota has been subject to speculation (Dawson, 1963; Dumbleton, 1967; Fleming, 1963; Heenan and McGlone, 2013; Raven, 1973; Wardle, 1978), but the consensus is that the formation of the Southern Alps preceded the alpine radiations.

Here we investigate a radiation of endemic New Zealand grasshopper (kōwhitiwhiti) species (Fig. 1). The 13 species, in four endemic genera (*Alpinacris, Brachaspis, Paprides, Sigaus;* (Bigelow, 1967; Hutton, 1897)), share characteristics that suggest they share a common ancestor that could tolerate cold environments (Bigelow, 1967). Ten species are clearly “alpine” in their distributions, physiology, morphology and ecology (Batcheler, 1967; Bigelow, 1967; Hawes, 2015; Sinclair, 2001), but three species (*Brachaspis robustus*, *Sigaus childi* and *Sigaus minutus*) are known only from the low elevation semi-arid zone of central South Island (Bigelow, 1967; Jamieson, 1999). Globally, grasshoppers inhabiting alpine/cold environments typically complete their lifecycle between spring and autumn (fall), overwintering as eggs (Alexander and Hilliard, 1964; Liu and Wang, 2003). However, New Zealand grasshoppers tolerate freezing at all life stages (Batcheler, 1967; Hawes, 2015; Mason, 1971; Sinclair, 2001) so can and do overwinter at any age, resulting in multiple egg clutches per season, relatively long lifespans and overlapping generations (Batcheler, 1967; Mason, 1971). Such a flexible physiology may reflect an adaptive response to New Zealand’s erratic and changeable climate, where alpine snowfall can occur at any time throughout the year and the longevity of snowpack is variable (Batcheler, 1967; Sinclair and Chown, 2005; Sturman and Wanner, 2001).

Using fossil calibrated phylogenies we test the expectation that the New Zealand alpine grasshopper fauna comprises a monophyletic group that diversified in New Zealand in response to the formation of the Southern Alps approximately 5 Mya. Current evidence suggests that at this time suitable open habitat became available, induced by topographic uplift (Fleming, 1963; Heenan and McGlone, 2013; Winkworth et al., 2005), that would have suited the basking and feeding habits of short-horn grasshoppers in temperate regions. We consider whether a single arrival of the lineage is sufficient to explain the origins of this grasshopper diversity despite taxonomy that recognises four distinct genera. Estimating stem age of such a clade is of course very sensitive to sampling of related taxa outside New Zealand (Crisp et al., 2011), so we included representatives of the geographically nearest and putatively closest relatives (Bigelow 1967) inhabiting the alpine zone outside New Zealand. We use six different fossils and whole mitochondrial genomes representing the global diversity of grasshoppers (Orthoptera: Caelifera) in order to test the hypothesis that the species radiation of New Zealand alpine grasshoppers coincided with orogeny of the Southern Alps.

2. Materials and methods

2.1. Taxa and sampling

The New Zealand alpine grasshopper species (subfamily Catantopinae) are morphologically and ecologically most similar to species in Tasmania, Australia (genera *Russalpia* and *Tasmaniacris*). The Tasmanian and New Zealand species live on mountains, are flightless, and lack the ability to communicate audibly (Bigelow, 1967; Key, 1991). Examination of male genitalia suggests New Zealand *Sigaus* could be sister to *Russalpia* (Bigelow, 1967), while New Zealand *Brachaspis* and *Paprides* resemble *Tasmaniacris* (Key, 1991). Other Acrididae that are adapted to the alpine habitat of mainland Australia include *Kosciuscola* and *Praxibulus* (Slatyer et al., 2014; Umbers et al., 2013). Although morphological evidence indicates these Oxyinae are not closely related to New Zealand taxa, they were included in our phylogenetic study for comparison. Thus, we sampled four Australian alpine species: the Catantopinae *Russalpia albertisi* Bolivar, 1898 and *Tasmaniacris tasmaniensis* Bolivar, 1898 from Tasmania, and Oxyinae *Kosciuscola cognatus* Rehn, 1957 and *Praxibulus* sp. from mainland Australia (Table 1).

The New Zealand fauna was sampled by selecting suitable species as representatives of known mtDNA lineages. Specifically, the endangered

species *Brachaspis robustus* Bigelow, 1967 and *Sigaus childi* Jamieson, 1999 are known to nest phylogenetically within *B. nivalis* (Trewick and Morris, 2008; Trewick, 2001) and *S. australis* (Dowle et al., 2014; Trewick, 2008) respectively, and so were unnecessary in the present analysis. Permits for this study were provided by the New Zealand Department of Conservation - Authorisation Number: 49878-RES. Data were obtained from 14 individuals representing eleven New Zealand species: *Alpinacris crassicauda* Bigelow, 1967; *Alpinacris tumidicauda* Bigelow, 1967; *Brachaspis collinus* Hutton, 1897; *Brachaspis nivalis* Hutton, 1897; *Paprides dugdali* Bigelow, 1967; *Paprides nitidus* Hutton, 1897; *Sigaus australis* Hutton, 1897; *Sigaus campestris* Hutton, 1897; *Sigaus minutus* Bigelow, 1967; *Sigaus piliferus* Hutton, 1897 and *Sigaus villosus* Salmon, 1950 (Fig. 1, Table 1). Three of these species were each sampled at two locations to encompass undocumented diversity indicated by preliminary data (Table 1). Species were identified using morphological traits, with the pronotum margin, subgenital plate and epicrot being particularly informative (Bigelow, 1967).

We sampled a taxonomically related lineage (*Phaulacridium*) that has endemic species in low elevation habitat of Australia and New Zealand. The two species included were *P. marginale* Walker, 1870 and *P. otagoense* Westerman and Ritchie, 1984. This lineage is not considered part of the alpine grasshopper group, but the New Zealand species are sympatric at low elevation with some alpine taxa and belong to the same subfamily (Catantopinae) as New Zealand and Tasmanian alpine grasshoppers (Sivyer et al., 2018). Additional data for comparison were obtained from GenBank using the search algorithm BLAST to optimise our sample (Altschul et al., 1990). Mitochondrial DNA sequences were acquired for representatives of 66 grasshopper species comprising 43 published and 20 novel Caeliferan mtDNA genomes, and three Ensiferan mtDNA genomes.

2.2. DNA sequencing

The natural enrichment of eukaryote cells with the relatively small mtDNA genome makes NGS approaches using high-throughput to generate numerous short anonymous sequences an effective way to assemble them. Similarly, the nuclear 45S Ribosomal (rRNA) cassette is highly replicated in the genome, with many tandem repeats and copies on multiple chromosomes (Richard et al. 2008). The cassette of three rDNAs (18S, 5.8S and 28S) and two Internal Transcribed Spacers (ITS1 and ITS2) is thus amenable to assembly from high-throughput NGS data (Vaux et al. 2017). Despite biparental inheritance the 45S Ribosomal cassette tends to be homogenised via concerted evolution (Nei and Rooney 2005). The rDNA regions of the cassette are highly conserved and so show a slow rate of nucleotide substitution that has been used to study deep phylogenetic relationships (Raué et al. 1988). In contrast, the ITS regions are not functionally constrained in the same way, and so have high substitution rates. ITS sequences can vary greatly even within species, and have been used alongside mtDNA in species and population analyses (Álvarez &Wendel 2003, Richard et al. 2008, Trewick 2001). We sequenced a third nuclear gene family, the histone proteins H3 and H4 that are adjacent to one another in the genomes of these grasshoppers.

We assembled a rich DNA sequence database including entire mtDNA genome sequences which have been shown to contain phylogenetic signal capable of resolution of lineages that diverged up to 300 Mya (Fenn et al., 2008; Song et al., 2015). To do this we used a Next Generation Sequencing (NGS) and bioinformatics approach. Insect DNA was extracted using a salting out method (Sunnucks and Hales, 1996; Trewick and Morgan-Richards, 2005) and quantified using Qubit fluorometry (Life Technologies, Thermo Fisher Scientific Inc.). Genomic DNA samples were paired-end sequenced through massive parallel, high-throughput sequencing on an Illumina HiSeq 2500 by Macrogen (http://dna.macrogen.com) following fragmentation and indexing using the Illumina TruSeq Nano DNA kit. Resulting 100 bp paired-end reads were sorted and edited using 'Cutadapt' v1.11 (Martin, 2011) to remove sample barcodes, and were paired and assembled in Geneious v9.1.4 (Kearse et al., 2012).

Mitochondrial genomes were obtained from each sample DNA using an iterative reference mapping approach. The first of the New Zealand grasshopper genome assemblies used a published annotated mtDNA genome of *Locusta migratoria* (Flook et al., 1995) for initial mapping. Paired reads were iteratively mapped to the reference sequence in Geneious generating a novel consensus sequence, which was then used as a reference to remap the raw sequence reads. This process was repeated until all alignment gaps were filled by extension with the new sequence data and ambiguities resolved. Henceforth subsequent assemblies began with the more similar reference templates from our first New Zealand grasshopper mtDNA genome. This approach has proved fast and efficient for a range of non-model organisms including birds (Garcia-R et al., 2014), and molluscs (Vaux et al., 2017). Sequences were uploaded as raw fasta files to MITOS (Bernt et al., 2013) for initial identification of protein coding regions, rDNAs and tRNAs. Annotations were transferred and individually cross-checked by comparison of reading frames, amino acid translation and RNA structure. Nuclear sequence data were treated in a similar manner to assemble, align and edit 45S Ribosomal cassettes and Histone (H3 and H4)) sequences for all 20 grasshopper species. Two shorthorn grasshopper 45S templates: *Locusta migratoria* (Genbank: KM853191) and a species of Gomphocerinae (GenBank: AY859546), were used as initial reference sequences for the Ribosomal cassette and *L. migratoria* (Genbank: AF370817) for H3 and H4.

2.3. Phylogenetic analysis

DNA sequence alignments were created using the MUSCLE (Edgar, 2004) tool in Geneious v9.1.4 and were checked and edited manually before being submitted to the Gblocks server (Castresana, 2000) to remove any remaining ambiguous characters. We used PartitionFinder2 (Lanfear et al., 2016) to identify appropriate gene partitions in the data and the most suitable models of DNA evolution for each, prior to analyses investigating phylogenetic relationships.

Phylogenetic hypotheses were inferred using Maximum Likelihood (ML) with RAxML v8 (Stamatakis, 2014) and Bayesian Inference (BI) with MrBayes v3.2.2 (Ronquist and Huelsenbeck, 2003). Maximum Likelihood analyses were conducted using 1000 random starting trees under a GAMMA model and GTR substitution matrix. Node support was assessed using 1000 non-parametric bootstrap iterations, applying fast bootstrapping, alongside a subsequent ML search. For BI, each analysis used a GTR substitution model combined with the proportion of invariable sites model Invgamma; models used 30 million generations with four MCMC chains in each, and a burnin of one million generations. Output statistics were analysed in Tracer v1.6 (Rambaut et al., 2015). Phylogenies were visualised in FigTree v1.4.3 (Rambaut, 2014).

Preliminary phylogenetic analyses used DNA sequence alignments of 26 taxa including representative Ensifera, and the Caeliferan superfamilies Tridactyloidea, Tetrigoidea, Eumasticoidea and Acridoidea for which whole mtDNA genome data were available via GenBank (Table 1). Nine newly sequenced representatives of New Zealand, Australian and Tasmanian grasshopper fauna were included (n = 35) in order to test the suitability of published representatives of the grasshopper family Pamphagidae as an outgroup for the subsequent monophyly analysis. Once the suitable outgroup was established, phylogenetic analyses used data alignments representing 43 international species of Acrididae plus all 20 of our novel mtDNA genomes for New Zealand/Australia/Tasmania species, and three members of Pamphagidae as an outgroup (n = 66). These allowed monophyly of the New Zealand alpine grasshoppers to be assessed, the closest relatives of New Zealand's endemic grasshoppers to be identified, and provided the phylogenetic basis for the time-calibrated analyses.

Table 2
Fossil Orthoptera: Caelifera used to calibrate divergence dating analyses of the New Zealand short-horn alpine grasshopper radiation, with details of geological source and age. Mya = Million years ago.

Fossil	Superfamily	Species	Notes	Median age (Mya)	Fossil horizon age span	Reference
1	Locustopsoidea	*Eolocustopsis primitiva*	Forewing (incomplete), forewing length = 17 mm. Changhsingian delta plain shale in South Africa.	255.7	251.0–260.4	Riek (1976)
2	Tridactyloidea	*Monodactyloides curtipennis*	Exoskeleton (whole insect), body length = 11.5 mm. Aptian lacustrine siltstone/marl in Russia.	131.15	129.4–132.9	Sharov (1968)
3	Tetrigoidea	*Prototetrix reductus*	Forewing (complete), forewing length = 6.2 mm. Aptian lacustrine siltstone/marl in Russia.	131.15	129.4–132.9	Sharov (1968)
4	Eumasticoidea	*Archaeomastax jurassicus*	Fore and hindwings, forewing length = 10 mm. Callovian/Oxfordian lacustrine siltstone in Kazakhstan.	154.25	145.0–163.5	Sharov (1968)
5	Acridoidea	*Tyrbula russelli*	Exoskeleton (complete female), body length = 23 mm. Chadronian lacustrine shale in Colorado.	35.95	33.9–38.0	Scudder (1885)
6	Acridoidea	*Menatacridium eocenicum*	A wing. Its type locality is Menat (Piton collection). Thanetian crater lake diatomite in the Menat Formation, France.	57.25	55.8–58.7	Piton (1936)

2.4. Divergence dating analysis

We assessed the timing of the New Zealand alpine grasshopper radiation using BEAST2 v2.4.4 (Bouckaert et al., 2014). To calibrate the phylogeny we used a whole mtDNA genome dataset including suitable lineage representatives and six fossil calibrations (Table 2). All have been previously trialled in divergence dating analysis of Orthoptera (Song et al., 2015; Song et al., 2018; Wolfe et al., 2016). As molecular clock analyses are sensitive to choice and placement of calibrations (Ho and Phillips, 2009; Sauquet et al., 2011) the employment of several different fossils is preferred because it enables internal rate verification and exploration of prior assumptions.

We used BEAUti2 v2.4.7 (Bouckaert et al., 2014) to generate.xml files implementing settings for BEAST2 to run a GTR site model, a Relaxed Log Normal clock model and a Birth Death model as the tree prior. A suite of divergence dating analyses were used to explore the data and compatibility of calibrations. This involved 102 BEAST2 runs implemented using permutations of 3 DNA alignments, 22 taxon sets and 24 fossil and stratigraphy calibration combinations. DNA alignments were either 1) complete mtDNA genomes (13 protein coding genes, 22 transfer RNAs and two ribosomal RNAs), 2) the 13 mtDNA protein coding genes and 2 RNAs, or 3) the 13 mtDNA protein coding genes alone. Taxa included representatives of Ensifera, Tridactyloidea, Tetrigoidea, Eumasticoidea, Pamphagidae and Acridoidea, plus either all 14 available New Zealand alpine grasshopper sequences, or a representative subset of 5 taxa (*A. crassicauda*, *B. nivalis*, *S. australis*, *S. campestris* and *S. piliferus*).

We examined the effects on divergence times, model convergence and Effective Sampling Size (ESS) scores of applying different combinations of the six fossils (Table 2) and age distribution priors (e.g. normal, log normal, exponential). All fossil calibration points were constrained by monophyly and age. We used the fossil *Eolocustopsis primitiva* Riek, 1976 from the Changhsingian (latest Permian) in Natal, South Africa to calibrate the Caelifera clade as it is amongst the earliest representatives of that orthopteran suborder (Wolfe et al., 2016). The Russian fossil *Monodactylus curtipennis* Sharov, 1968 from the Aptian (Early Cretaceous) was used to constrain the Tridactyloidea clade. *Prototetrix reductus* Sharov 1968 from the same sampling site, was used to calibrate the Tetrigoidea clade as it is the oldest definitive fossil known for this group. Similarly, *Archaeomastax jurassicus* Sharov 1968 from the Callovian/Oxfordian (Middle Jurassic) was used to calibrate the Eumasticoidea clade as it is the oldest known fossil for this group. Two fossils were used separately to calibrate the large ingroup clade, Acridoidea - *Tyrbula russelli* Scudder, 1885 and *Menatacridium eocenicum* Piton, 1936. *Tyrbula russelli* is a representative of New World Acrididae, from the Eocene Florrisant beds in Colorado, USA, whereas *Menatacridium eocenicum* is interpreted as representing Old World Acrididae, from the Thanetian (Early Eocene) in the Menat formation, France (Song et al., 2018). Both have previously been used to calibrate the Acrididae clade (Song et al., 2015; Song et al., 2018), but their respective influence on timing is tested here.

Time estimated phylogenies were obtained using BEAST2 analyses run for either 10 or 100 million generations, sampling every 1000 or 10,000 generations, respectively. Tracer was used to investigate the Bayesian outputs, and ESS statistics (prior, posterior, tree likelihood, tree height) were used to indicate whether the posterior space of the models was sufficiently explored. ESS values > 200 are considered sufficient for the analyses to be informative (Heath, 2015; Ogilvie et al., 2016). Maximum clade credibility trees with median heights were generated in TreeAnnotator v2.4.4 (Bouckaert et al., 2014) and edited in FigTree, where estimated dates of divergence and their 95% HPD intervals could be visualized. The processing power of the CIPRES Science Gateway v3.3 (Miller et al., 2010) was used for all phylogenetic tree construction and divergence dating analyses.

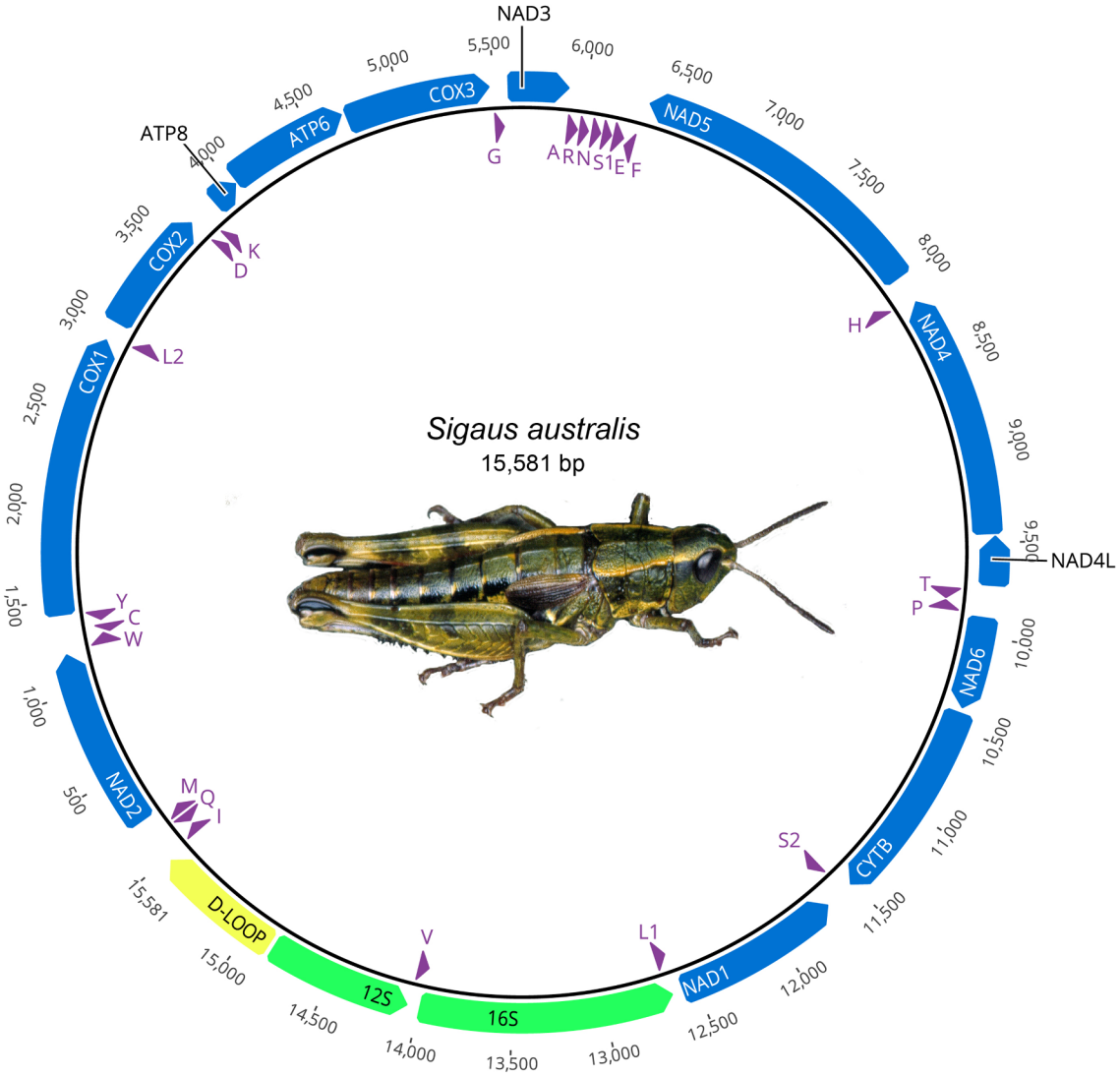

Fig. 2. The mitochondrial genome composition of the New Zealand grasshopper *Sigaus australis*, 20 whole mitochondrial genomes were sequenced for this study. Protein coding genes are coloured in blue, tRNA genes are coloured in purple, rDNA genes are coloured in green and the A + T rich repeat region is coloured in yellow. Arrows at the ends of annotation bars indicate the gene direction. Transfer RNA genes are labelled using IUPAC-IUB single letter amino acid codes (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Novel DNA sequence data

Complete mtDNA genome sequences were assembled for 20 grasshopper individuals. The total number of 100 bp paired reads obtained for these taxa ranged from 9,098,272 (*S. campestris* GH1036) to 26,413,966 (*Ph. marginale* GH762), of which a small (0.0005% *P. dugdali* GH830 to 0.006% *T. tasmaniensis* TAZ4) but sufficient proportion mapped to the mtDNA reference sequence. The paired read coverage of mtDNA varied among samples, ranging from 5,951 (*P. dugdali* GH830) to 67,214 (*T. tasmaniensis* TAZ4) in number with mean depth of paired reads per sample having a ten-fold range (55 *S. campestris* GH1036 to 526.8 *T. tasmaniensis* TAZ4) in sequences (Supplementary Table S1). Assembled mtDNA genomes ranged in length from 15,468 base pairs (bp) (*S. piliferus* LW GH5) to 15,837 bp (*S. villosus* GH1374), with all mtDNA genomes comprising the expected arrangement of 13 protein coding genes, 22 tRNAs, two rDNAs and a repeat region (D-loop) (Fig. 2) (Cameron, 2014; Fenn et al., 2008; Flook et al., 1995; Ma et al., 2009; Zhou and Huang, 2016). The order and orientation of genes was the same for all 20 individuals and identical to that of other Acrididae for which data are available (Fenn et al., 2008; Flook et al., 1995; Ma et al., 2009; Song et al., 2015; Zhou and Huang, 2016). Variation in genome length was mainly attributable to differences in length of the D-loop and intergenic regions. Overall GC content of the mtDNA genomes was low, ranging from 25.3% (*S. piliferus* GH60) to 27% (*S. australis* GH1387).

The combined length of the 13 concatenated protein coding genes varied from 11,139 bp (*P. dugdali* GH830) to 11,151 bp (*S. campestris* GH1036, *S. minutus* GH433, *S. piliferus* MH GH60 and *R. albertisi* TAZ3), reflecting differences in number of amino acids. *Sigaus villosus* (GH1374) had two additional amino acids at ND5 compared to others, while the ND4L gene of *T. tasmaniensis* (TAZ4) was two amino acids shorter than all others. *Sigaus campestris* (GH1036), *S. minutus* (GH433), *S. piliferus* (GH5; GH60), *S. villosus* (GH1374) and *R. albertisi* (TAZ3) all had an amino acid insertion in ND6 compared to the others, and *S. piliferus* (GH5) and *S. villosus* (GH1374) each lacked the penultimate codon of CYTB compared to others. The concatenated rDNA regions (12S and 16S) ranged in length from 2138 bp (*S. villosus* GH1374) to 2180 bp (*S. piliferus* GH5), while concatenated tRNA length varied from 1473 bp (*A. crassicauda* GH749) to 1483 bp (*T. tasmaniensis* TAZ4). Intergenic regions were found to account for no more than 0.8% of total

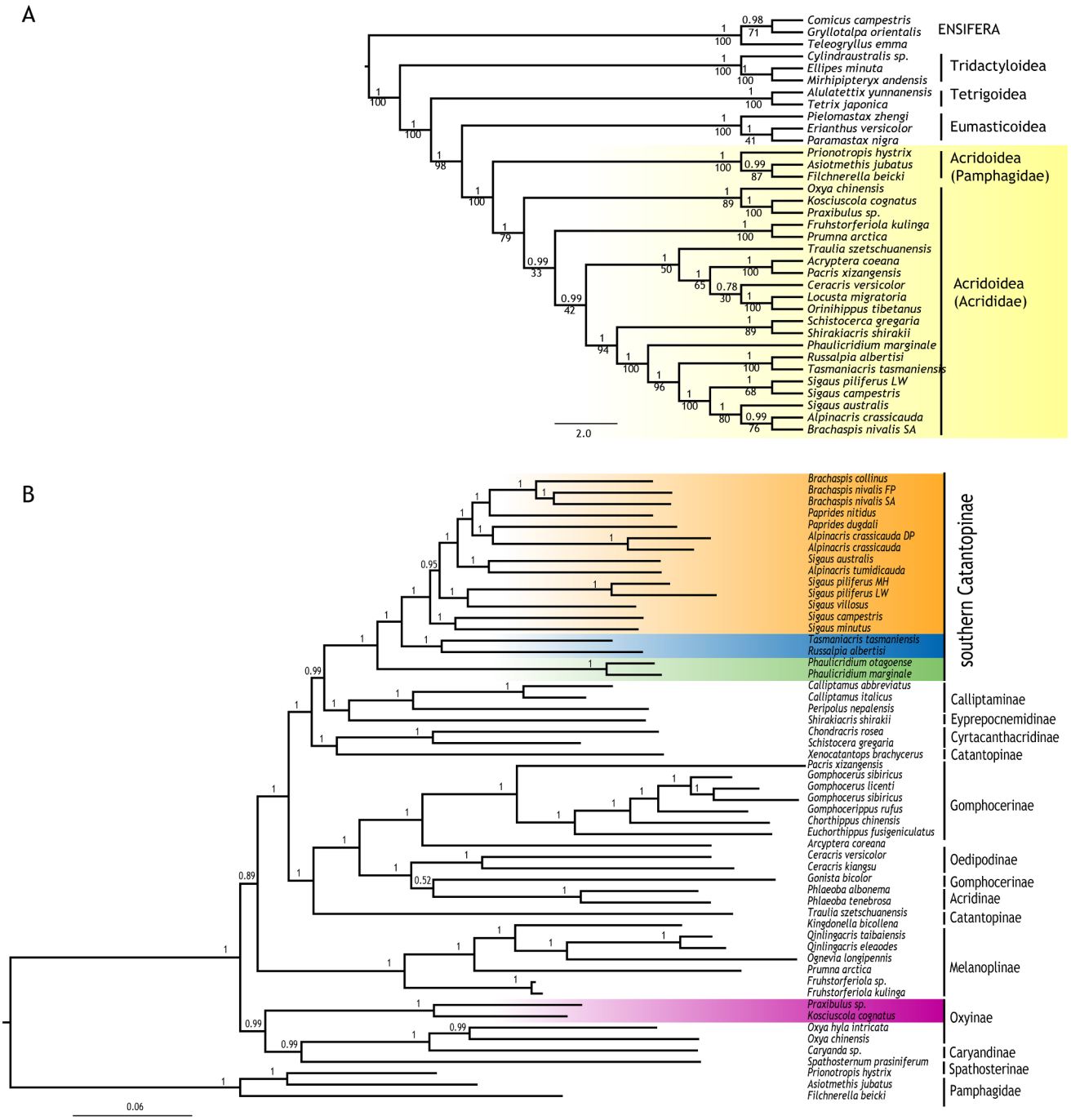

Fig. 3. Grasshopper evolutionary relationships inferred from whole mitochondrial genome sequence. (A) Phylogenetic hypotheses to identify suitable outgroups for Acrididae. Bayesian Inference (BI) and Maximum Likelihood (ML) phylogenetic trees of mitochondrial genome alignments using 10,009 bp alignment of protein coding gene data for 35 Orthoptera. Three Ensiferans were enforced as the outgroup. BI posterior probabilities and ML bootstraps (1000 bootstraps) are above and below node edges, respectively. Yellow box highlights sister lineages of Caelifera Acrididae and Pamphagidae used in subsequent analyses. (B) Phylogenetic hypothesis of Acridoidea grasshopper relationships inferred from mitochondrial DNA using a Maximum Likelihood (ML) analysis. This 13,878 bp alignment of 55 Acrididae mitochondrial genome sequences consisted of protein coding, tRNA and rRNA gene data. Three Pamphagidae sequences were enforced as the outgroup. BI posterior probabilities are shown at nodes. New Zealand's endemic alpine grasshoppers are highlighted in orange, New Zealand's endemic lowland grasshoppers in green, alpine grasshoppers from Tasmania in blue, alpine grasshoppers from mainland Australia in mauve. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

genome lengths. Gene overlap of 7 bp was evident between ATP6 and ATP8, and between ND4 and ND4L. This is consistent with published mtDNA genomes of other Acrididae (Fenn et al., 2008; Flook et al., 1995; Ma et al., 2009; Song et al., 2015; Zhou and Huang, 2016).

45S rRNA cassettes were assembled for all New Zealand taxa plus the two alpine species from Tasmania and two alpine species from Australia. The resulting alignment of 20 grasshopper individuals showed high conservation at 18S (45/1967 variable sites), 5.8S (0/160) and 28S (113/4264) and as expected greater variation including many INDELs at ITS1 (219/410) and ITS2 (118/223). The alignment of two histone genes, H3 and H4, that are in close proximity with an intergenic spacer of about 260 bp comprised variation at 72 of 723 positions (4/241 AA).

3.2. Phylogenetic analysis

After the removal of gaps and ambiguities the mitochondrial genome alignment lengths for the outgroup test and monophyly analysis were 10,009 bp and 13,878 bp respectively. ML and BI trees were identical in topology for the outgroup dataset (Fig. 3a) and were consistent with previous studies (Song et al., 2015). After establishing Pamphagidae as a suitable Caeliferan outgroup to the sample of Acrididae, we examined monophyly of the New Zealand alpine grasshoppers using a broad sampling of taxonomic representatives including several putative Catantopinae. ML and BI trees were similar in topology, differing only in a polytomy of branches deep in the BI tree (Fig. 3b). Forty-one out of 62 nodes had Maximum Likelihood Bootstrap (MLB) and Bayesian Inference Posterior Probability (BIPP) values of 100 and 1 respectively. The four New Zealand alpine genera of interest (*Alpinacris*, *Brachaspis*, *Paprides* and *Sigaus*) formed a well-supported monophyletic clade (ML 100, BIPP 1). The two Tasmanian species (*T. tasmaniensis* and *R. albertisi*) formed a clade sister to the New Zealand alpine genera (ML 90, BIPP 1) consistent with inferences from morphology. Two other putative Catantopinae, *Xenocatantops brachycerus* and *Traulia szetschuanensis* from China, are shown to be misplaced in this subfamily and more closely allied to Gomphocerinae. The representatives of the Australasian low elevation genus *Phaulacridium* were sister to the clade of New Zealand and Tasmanian alpine grasshoppers (ML 100, BIPP 1) within the Catantopinae. Two other mainland Australian alpine grasshoppers sequenced for this study (*Kosciuscola cognatus* GH1721 and *Praxibulus* sp. GH1722) grouped within the Oxyinae clade with *Oxya chinensis* and *Oxya hyla intricata* (MLB 72, BIPP 0.99).

Phylogenetic analyses of the two sets of nuclear sequence data separately and concatenated, and with/without the ITS regions of the 45S cassette, resulted in topologies consistent with the better resolved mtDNA trees (Fig. 4 inset). The low overall level of sequence variation and high proportion of INDELS limited phylogenetic resolution, and equivalent data at this scale are not available for other grasshoppers. However, we found that the Australian Oxyinae were sister to the Catantopinae in our data. Within our sample of Catantopinae *Phaulacridium* was sister to the alpine species, as identified using mtDNA genome data, and the nuclear sequence corroborated the inference of monophyly of the New Zealand alpine grasshopper species.

3.3. Divergence dating analysis

Initial analyses with different combinations of calibrations and prior distributions were used to optimise data and taxon representation based on an evaluation of Effective Sampling Size (ESS) statistics in Tracer (Table 3). During this process of optimising the datasets, it was recognised that the Acridid Oedipodinae clade generated rate discrepancies with other taxa and was removed from BEAST2 run 42 and subsequent analyses. It was also determined that the most stable analyses resulted when using just protein coding genes, which were solely used from BEAST2 run 25 onwards (Table 3). Initially, normal distribution priors were used on fossil calibrations, however we also explored the effects of applying uniform (e.g. run 54), exponential (e.g. run 55), and log normal (e.g. run 80) distributions. In two analyses (runs 79 and 84), a combination of prior distributions were applied to different fossil calibrations, with a normal prior on fossils 1, 2 and 4 (Table 2) and exponential or log normal on fossil 5 (Table 3). For analyses using an exponential prior (runs 73, 74, 88 and 89) values for the means were varied. Some model permutations did not converge during MCMC (e.g. runs 53 and 78) or yielded very low effective sample size values (e.g. runs 5, 6, 10). Across all 102 BEAST2 runs, the median age inferred for the most recent common ancestor of the New Zealand and Tasmanian taxa was 21.79 Mya (mean age 24.25 Mya, SD = 10.04), and the median age of the most recent common ancestor of the New Zealand alpine grasshoppers was 18.97 Mya (mean age 21.09 Mya, SD = 9.29).

We present time calibrated trees with high ESS scores (Figs. 4 & 5), but note that the inferred age of the New Zealand alpine grasshopper radiation was not sensitive to either the priors or combination of fossil calibrations used. Molecular clock analysis using an alignment of 13 concatenated mtDNA protein coding genes (10,009 bp) and 27 taxa including five New Zealand alpine representatives (run 72, Fig. 4) had a topology and time scale consistent with analysis of an alignment of mtDNA protein coding genes for 32 grasshoppers including 14 New Zealand alpine grasshopper specimens (run 56, Fig. 5). These were typical of results regardless of the number and combination of fossils and their respective prior models. With an alignment including five representatives of the New Zealand alpine grasshopper fauna among a total of 27 grasshoppers (run 72), using four fossil calibrations (*Eolocustopsis primitive*, *Monodactyloides curtipennis*, *Archaeomastax jurassicus*, *Tyrbula russelli*) and a normal prior distribution (Fig. 4), we inferred that the most recent common ancestor of the Tasmanian and New Zealand alpine grasshoppers lived about 22 million years ago, (22.36 Mya; 95% HPD = 16.74–27.47 Mya) and the New Zealand alpine species had a common ancestor between 13 and 24 million years ago (19.21 Mya: 95% HPD = 13.66–24.41 Mya).

Separate analysis of the mtDNA protein coding gene alignment included all 14 New Zealand grasshopper taxa (run 56) among a total of 32 grasshoppers, one fossil calibration (*Tyrbula russelli*) and an exponential distribution prior (Fig. 5). This resulted in a tree with topology compatible with other analyses and only minor differences in the divergence dates estimated using multi-fossil calibrations. The most recent common ancestor of the Tasmanian and New Zealand alpine grasshoppers was estimated to have lived about 19 million years ago, (18.68 Mya; 95% HPD = 15.06–24.19 Mya) and the New Zealand alpine species had a common ancestor between 13 and 22 million years ago (16.85 Mya; 95% HPD = 13.55–21.8 Mya).

Replicating analyses with one or more fossil calibrations but replacing Acrididae fossil *Tyrbula russelli* (fossil 5) with *Menatacridium eocenicum* (fossil 6) yielded earlier node ages for the ingroup Acrididae, as expected from the fossil age. For example, with protein coding data for 32 specimens, calibration with *Tyrbula russelli* gave 22.33 Mya (15.3–25.14) and 20.46 Mya (13.65–22.53) for Tasmanian/NZ ancestor and NZ radiation respectively whereas *Menatacridium eocenicum* gave 31.46 Mya (26.96–35.47) and 28.09 Mya (23.98–21.9) (Table 3).

To further test the hypothesis that the New Zealand alpine grasshopper radiation coincided with mountain building activity in New Zealand, we ran four analyses with a 'mountain building' calibration prior (e.g. BEAST2 runs 58, 59, 78 and 85; Table 3) at 5 Mya ± 2.5 My. Three of these analyses also included fossil calibration priors and resulted in reduced node ages (c.f. median node ages across the 102 BEAST2 runs), however, ESS and node posterior probabilities declined. When the mountain building calibration was used by itself, the model failed to converge, indicating incompatibility with the DNA sequence variation.

Finally, we extracted mean DNA substitution rates from the optimal molecular clock analyses in BEAST2. This yielded site rates of 4.04E-03 (Run 72, Fig. 4) and 5.94E-03 (Run 56, Fig. 5) equivalent to divergence rates of 0.81% per million years and 1.19% per million years for an alignment comprising all mitochondrial coding genes.

4. Discussion

The rich fauna and flora of alpine adapted species in Aotearoa has long fascinated biologists intrigued by their apparent rapid pace of evolution (Buckley and Simon, 2007; Dumbleton, 1967; Fleming, 1963; Raven, 1973; Wardle, 1978; Winkworth et al., 2005). The geological youth of the mountain environment in which these alpine specialists exist leads to an inference of recent adaptation or recent arrival once suitable habitat became available (Heenan and McGlone, 2013). However, our fossil calibrated analysis of New Zealand Acrididae

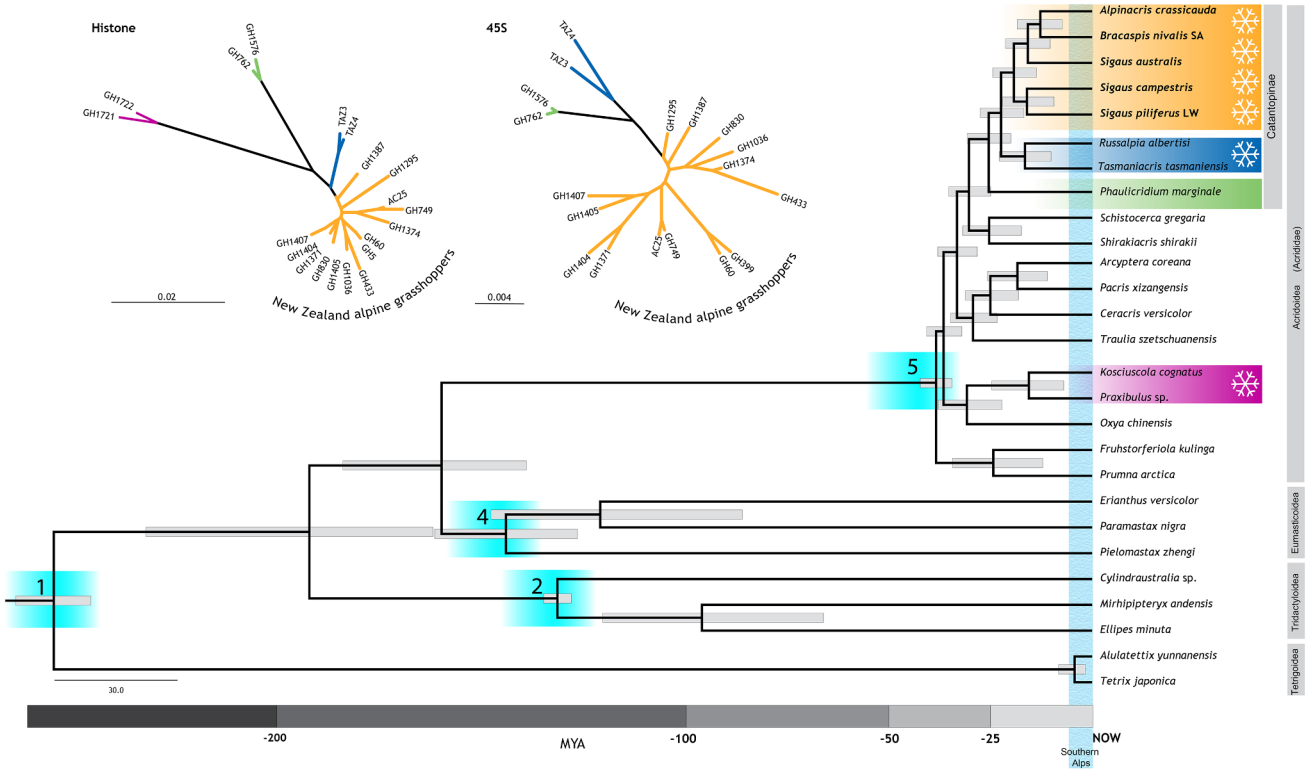

Fig. 4. Phylogenetic hypothesis of Acridoidea relationships and divergence dates inferred from mitochondrial DNA using Bayesian Inference (BI). This alignment consisted of 27 Acrididae mitochondrial genome sequences and was 10,009 bp in length. No sequences were enforced as the outgroup. New Zealand's endemic alpine grasshoppers are highlighted in orange, New Zealand's endemic lowland grasshoppers in green, alpine grasshoppers from Tasmania in blue, alpine grasshoppers from mainland Australia in mauve. Fossil calibrations are indicated in turquoise at nodes: 1. *Eolocustopsis primitiva*, 2. *Monodactylus curtipennis*, 4. *Archaeomastax jurassicus*, 5. *Tyrbula russelli* (Table 2) with normally distributed prior for each (Run 72 in Table 3). Inset networks represent results from analysis of nuclear DNA sequences for Histone H3 and H4, and the 45S rRNA cassette (18S, ITS1, 5.8S, ITS2, 28S) using a neighbour-joining analysis with Tamura-Nei distances and maximum likelihood analysis with the HKY model of DNA evolution, respectively. These are just two examples of consistent topological results with respect to monophyly of New Zealand taxa and their sister relationship to Tasmanian alpine species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

indicates diversification a considerable time (~12 My) prior to the orogenic activity that gave rise to the dominant mountain terrain in New Zealand (Batt and Braun, 1999; Davey et al., 2007; Kamp et al., 1989; Kamp and Tippett, 1993; Koons, 1989). This finding not only runs counter to inferences about the role of mountain formation in other New Zealand species radiations but diversification of montane grasshopper lineages more widely. For instance, speciation of *Melanoplus* in North America (Cigliano and Amedegnato, 2010; Knowles, 2001) and *Jivarini* in South America (Cigliano and Amedegnato, 2010) are interpreted as coinciding with mountain formation.

Within our sampling of grasshoppers we confirmed the sister relationship between New Zealand and Tasmanian alpine grasshoppers predicted by their morphology (Bigelow, 1967; Key, 1991). The last common ancestor of this clade was estimated to have lived about 20 Mya (Miocene), from which we can infer at least one ancestral dispersal and colonisation event across the Tasman Sea (Fig. 1). This is long after separation of the Gondwanan continental fragments from which Australia (including Tasmania) and New Zealand are derived, and after the period of maximum marine inundation of Zealandia that preceded formation of New Zealand (Mortimer et al., 2017; Trewick et al., 2007). A continental vicariance explanation for the *trans*-Tasman sister relationship as mooted by Bigelow (1967) is therefore not supported. Grasshoppers in this lineage can be added to the many examples of plants (Winkworth et al., 2005), invertebrates (Goldberg et al., 2015; Goldberg and Trewick, 2011), lizards (Nielsen et al., 2011) and birds (Trewick and Gibb, 2010), for which colonisation of New Zealand via dispersal is supported (Buckley et al., 2010; Mildenhall, 1980; Nielsen et al., 2011; Wallis and Jorge, 2018; Winkworth et al., 2005). Less likely is colonisation east to west against the circumpolar circulation that have prevailed for > 30 million years (Parker et al., 2007).

During time-calibration of the grasshopper phylogeny we found that the six available fossils were not all compatible within a single analysis, but we found internal support for a combination of four fossils ranging in age from 256 to 36 million years (Table 2). The estimated age of the last common ancestor of the New Zealand alpine grasshoppers proved resilient across a range of model priors suggesting that the analyses yielded reliable inferences. Our estimates of DNA substitutions rates from all mitochondrial coding genes of 0.81% to 1.19% per million years is not usual for the time depth (> 250 million years), and compatible with estimates based on shallower clades and shorter markers (e.g. using stratigraphic calibration Goldberg et al. (2014) inferred a divergence rate for mtDNA cytochrome oxidase subunit 1 in New Zealand carabid beetles of 1.18% per million years). Influential in understanding ingroup age were two fossils that have each been considered to be the oldest representatives of the Acrididae; *Menatacridium eocenicum* from Paleocene in France 58.7–55.8 Mya, and *Tyrbula russelli* from Chadronian lacustrine shale in Colorado 33.9–38.0 Mya (Table 2). Recently when analysing concatenated DNA sequences of Caelifera (short-horn grasshoppers), Song et al. (2018) proposed that the geographic occurrence of *M. eocenicum* in Europe and deep phylogenetic placement of Neotropical subfamilies rendered it unlikely to represent stem Acrididae. If this interpretation is correct, the base of the Acrididae is likely to be older than implied by use of *T. russelli* and indeed Song et al. (2018) estimated this family originating 59.3 Mya. For the present analysis, this renders it unlikely that our estimate of the time of ingroup radiation is excessive (i.e. too old). Supporting this was the

Table 3

Improvement of dating confidence through iterative analysis of combination of DNA sequence partitions and taxon sampling 102 BEAST2 analyses investigated for this study. NZ Species notes if run contained all New Zealand (NZ) species or just five representatives (Reps). Calibration numbers refer to fossils in Table 2, whilst G represents Southern Alps orogeny at 5Mya. The Alignment type column notes whether the sequences consisted of protein coding genes (CDS), ribosomal RNA genes (rRNA) and/or transfer RNA (tRNA) genes. Effective Sample Size (ESS) summary statistics for Posterior, Prior and Tree Likelihood are provided with values > 200 in bold. Prior distributions on calibration ages were: N normal (the variance of the normal distribution σ = 1 unless indicated otherwise), E exponential ($\bar{x}$ = 5 unless indicated otherwise), or LN lognormal. Divergence dates (millions of years) are given as posterior density median (node age) of the Tasmania (Tas)/ New Zealand (NZ) split and the NZ crown group are displayed, along with their respective Highest Posterior Density interval (HPD) values. Two BEAST2 runs (72 & 56) are illustrated (Figs. 4 & 5).

BEAST2 Run	NZ Species	Number of taxa included	Calibration	Alignment type	Prior distribution	Number of generations	Posterior	Prior	Tree Likelihood	Tree Height	Tas/NZ split age	Tas/NZ 95% HPD	NZ crown group age	NZ 95% HPD
1	All	53	5	CDS, rRNA, tRNA	N	10	12	**233**	12	72	19.96	16.35;23.82	17.71	14.45;21.27
2	Reps	44	5	CDS, rRNA, tRNA	N	10	138	35	53	91	19.33	14.59;24.16	16.92	12.44;21.70
3	All	56	2,5	CDS, rRNA, tRNA	N	10	15	47	15	**210**	20.81	17.18;24.89	18.61	15.52;22.26
4	Reps	47	2,5	CDS, rRNA, tRNA	N	10	13	67	11	64	20.92	14.96;24.91	18.57	13.23;22.68
5	All	55	3,5	CDS, rRNA, tRNA	N	10	4	15	5	6	25.87	21.24;30.92	22.72	14.76;30.58
6	Reps	46	3,5	CDS, rRNA, tRNA	N	10	3	6	3	15	21.72	13.40;26.48	16.49	8.37;20.83
7	All	58	2,3,5	CDS, rRNA, tRNA	N	10	115	26	**844**	7	24.33	18.37;29.42	21.92	15.98;27.02
8	Reps	59	2,3,5	CDS, rRNA, tRNA	N	10	16	23	13	10	14.95	11.74;20.74	13.41	9.64;19.77
9	All	61	2,3,4,5	CDS, rRNA, tRNA	N	10	4	12	4	21	25.30	17.93;31.04	21.83	16.46;29.44
10	Reps	52	2,3,4,5	CDS, rRNA, tRNA	N	10	4	9	4	4	12.85	5.14;27.35	9.58	3.55;20.71
11	All	64	1,2,3,4,5	CDS, rRNA, tRNA	N	10	9	6	9	165	21.85	16.1;31.44	19.84	12.33;28.33
12	Reps	55	1,2,3,4,5	CDS, rRNA, tRNA	N	10	4	4	4	74	19.00	10.71;25.27	14.35	8.73;19.47
13	All	53	5	CDS, rRNA	N	10	7	46	6	132	20.22	16.54;24.05	17.83	14.54;21.11
14	Reps	44	5	CDS, rRNA	N	10	**218**	**507**	189	149	19.31	15.54;22.98	17.18	13.48;20.76
15	All	56	2,5	CDS, rRNA	N	10	5	11	5	63	22.34	15.43;29.84	19.91	10.52;25.34
16	Reps	47	2,5	CDS, rRNA	N	10	38	22	71	49	18.86	13.28;26.19	15.37	9.63;20.99
17	All	55	3,5	CDS, rRNA	N	10	23	103	22	**320**	20.72	17.39;24.59	18.45	15.14;22.00
18	Reps	46	3,5	CDS, rRNA	N	10	31	128	22	**296**	20.34	16.26;24.99	18.16	13.89;22.56
19	All	58	2,3,5	CDS, rRNA	N	10	17	41	17	13	26.07	22.41;31.24	24.14	20.56;29.73
20	Reps	59	2,3,5	CDS, rRNA	N	10	10	32	10	25	21.21	14.74;29.22	17.37	11.40;23.96
21	All	61	2,3,4,5	CDS, rRNA	N	10	3	27	3	5	23.83	18.98;28.75	20.66	16.21;26.46
22	Reps	52	2,3,4,5	CDS, rRNA	N	10	3	10	3	10	23.07	14.56;30.42	18.87	11.45;26.99
23	All	64	1,2,3,4,5	CDS, rRNA	N	10	4	9	4	94	23.40	19.17;30.63	20.79	17.1;27.07
24	Reps	55	1,2,3,4,5	CDS, rRNA	N	10	5	14	5	23	16.70	11.97;23.35	13.23	7.92;20.59
25	All	53	5	CDS	N	10	86	**500**	85	162	20.66	17.26;24.05	18.56	15.41;21.75
26	Reps	44	5	CDS	N	10	**1037**	**502**	**988**	64	19.07	15.28;23.26	16.85	13.14;20.68
27	All	56	2,5	CDS	N	10	3	24	3	35	22.90	14.54;27.70	20.82	14.13;26.15
28	Reps	47	2,5	CDS	N	10	18	26	21	20	19.63	12.99;29.06	16.03	10.54;25.81
29	All	55	3,5	CDS	N	10	15	63	14	180	20.79	17.65;23.95	18.81	15.63;21.79
30	Reps	46	3,5	CDS	N	10	188	138	**266**	179	20.38	16.29;24.02	18.10	14.45;21.49
31	All	58	2,3,5	CDS	N	10	15	41	11	6	25.84	22.13;29.98	23.50	19.99;27.99
32	Reps	59	2,3,5	CDS	N	10	18	10	53	34	18.15	12.06;23.46	14.88	10.17;19.63
33	All	61	2,3,4,5	CDS	N	10	7	8	7	34	25.43	18.88;31.35	22.61	16.13;28.57
34	Reps	52	2,3,4,5	CDS	N	10	11	8	11	9	22.96	16.35;29.99	19.89	14.68;28.11
35	All	64	1,2,3,4,5	CDS	N	10	6	25	6	17	25.17	20.30;30.09	22.28	18.06;27.65
36	Reps	55	1,2,3,4,5	CDS	N	10	14	10	16	8	17.04	10.56;21.29	13.74	7.25;18.44

(*continued on next page*)

Table 3 (*continued*)

BEAST2 Run	NZ Species	Number of taxa included	Calibration	Alignment type	Prior distribution	Number of generations	Posterior	Prior	Tree Likelihood	Tree Height	Tas/NZ split age	Tas/NZ 95% HPD	NZ crown group age	NZ 95% HPD
37	Reps	53	5	CDS	N	100	**240**	**1791**	**265**	**925**	19.04	14.94;23.31	16.84	12.79;20.99
38	Reps	53	5	CDS	N	10	**383**	**194**	**633**	**8434**	16.21	12.82;20.03	14.41	11.18;18.02
39	Reps	53	4	CDS	N	100	**379**	**994**	**336**	**89,113**	16.21	12.45;20.29	14.40	10.89;18.25
40	Reps	56	2,4	CDS	N	100	**2676**	**634**	**4487**	**634**	18.24	14.64;22.00	16.29	12.74;19.88
41	All	53	4	CDS	N	10	**543**	161	**1141**	**9001**	16.92	13.67;19.94	15.28	12.39;18.09
42	Reps	44	4	CDS	N	10	**897**	140	**1887**	**8948**	16.59	13.19;19.96	14.89	11.70;18.10
43	All	43	4	CDS	N	10	**867**	**283**	**1274**	**9001**	21.26	17.29;25.22	19.08	15.49;22.71
44	Reps	34	4	CDS	N	10	**1139**	**230**	**1789**	**8423**	20.82	16.64;25.02	18.67	14.50;22.45
45	Reps	37	4,5	CDS	N	10	**392**	64	**1154**	49	20.93	16.88;24.92	18.61	14.72;22.92
46	Reps	36	3,5	CDS	N	10	178	66	81	54	19.52	12.86;26.79	15.68	7.43;24.18
47	Reps	42	1,3,4	CDS	N	10	**255**	57	**833**	59	21.05	16.76;25.42	18.23	14.48;22.31
48	Reps	42	1,4	CDS	N	10	**209**	72	**1302**	52	18.62	14.10;22.94	16.27	12.54;20.56
49	Reps	30	1,4	CDS	N	10	**433**	89	**947**	35	22.40	16.87;26.93	19.20	14.71;23.42
50	Reps	30	1,3,4	CDS	N	10	**229**	75	**1184**	60	22.59	17.10;28.14	19.12	13.44;25.09
51	Reps	27	1,4	CDS	N	10	**373**	101	**905**	**4692**	20.91	16.08;26.17	18.18	13.88;23.14
52	Reps	27	1,3,4	CDS	N	10	**692**	**264**	**750**	**3682**	21.27	16.59;26.77	18.43	13.50;23.19
53	All	32	5	CDS	N	10	105	101	**1285**	**816**	–	–	–	–
54	Reps	27	2,4	CDS	U	100	**4512**	**684**	**9524**	**59,174**	19.46	14.97;23.87	16.85	12.39;21.13
55	Reps	27	2,5	CDS	E	100	**5724**	**2359**	**10,120**	**6274**	19.98	14.81;25.01	17.38	12.4;22.31
56	**All**	**32**	**5**	**CDS**	**E**	**100**	**6772**	**3164**	**15,577**	**1930**	**18.86**	**15.06;24.19**	**16.85**	**13.55;21.8**
57	Reps	30	2,5	CDS	E	100	**6071**	**1770**	**9764**	**379**	20.99	16.35;25.72	18.29	14.03;23.00
58	Reps	27	2,5,G	CDS	N	10	145	48	**1511**	**5765**	9.78	6.21;14.98	6.64	5.04;8.44
59	All	32	5,G	CDS	N	10	166	73	**681**	46	8.64	6.57;10.91	7.47	5.83;9.14
60	Reps	27	1,5	CDS	N	10	10	**233**	9	**282**	20.50	16.26;27.50	17.83	14.0;22.01
61	Reps	30	4,5	CDS	E	10	7	3	6	5	77.12	0.56;50.29	72.17	0.25;44.75
62	Reps	30	2,4,5	CDS	E	10	6	5	6	34	34.63	2.35;47.58	31.10	2.80;42.75
63	Reps	30	2,5	CDS	E	10	6	6	6	11	22.71	7.16;34.96	18.71	4.99;30.38
64	Reps	30	1,5	CDS	E	10	23	21	24	**8914**	61.73	21.12;47.92	56.78	17.03;41.65
65	Reps	30	1,2,3,4,5	CDS	E	10	3	3	3	73	34.94	1.05;90.6	20.48	0.73;77.5
66	Reps	27	1,5	CDS	N	10	105	29	**1421**	**5780**	21.25	15.54;26.32	18.46	12.97;23.81
67	Reps	27	1,4,5	CDS	N	10	**359**	118	**743**	**3925**	22.95	17.77;27.72	19.77	15.13;24.78
68	Reps	27	1,4,5	CDS	E	10	3	4	3	**1380**	34.21	4.31;48.65	30.00	3.41;42.69
69	Reps	30	1,2,3	CDS	N	10	3	3	3	**575**	58.53	0.7;48.71	50.90	0.34;42.55
70	Reps	27	1,2,4,5	CDS	N	10	**424**	**220**	**998**	**3960**	23.90	19.35;28.37	20.88	16.26;25.04
71	Reps	27	1,2,4,5	CDS	E	10	7	6	7	**733**	27.62	4.64;37.88	24.03	1.96;32.15
72	**Reps**	**27**	**1,2,4,5**	**CDS**	**N**	**100**	**2585**	**958**	**7858**	**34,569**	**22.36**	**16.74;27.47**	**19.21**	**13.66;24.41**
73	Reps	27	1,2,4,5	CDS	E ($\bar{x}$ = 30)	10	4	4	4	35	31.93	1.03;44.64	27.67	0.56;39.43
74	Reps	27	1,2,4,5	CDS	E ($\bar{x}$ = 20)	10	4	4	4	95	31.76	1.87;47.61	26.30	1.42;42.1
75	Reps	27	1,2,4	CDS	N	10	18	27	15	**3092**	35.14	24.9;47.33	29.88	20.73;39.86
76	Reps	27	1,2,4,5	CDS	N (σ = 10)	10	7	20	7	**3652**	29.22	21.24;37.36	25.00	17.64;32.60
77	Reps	27	1,2,4,5	CDS	N (σ = 10)	10	175	70	**602**	**1184**	30.18	22.9;39.08	26.48	19.76;35.34
78	Reps	27	G	CDS	N	10	37	36	159	33	–	–	–	–
79	Reps	27	1,2,4,5	CDS	N(1,2,4),E(5)	10	178	25	**1067**	**4385**	33.24	23.55;44.34	28.85	20.21;39.2
80	Reps	27	1,2,4,5	CDS	LN	10	4	3	4	**1839**	2.14	0.55;22.02	1.38	0.36;17.95
81	Reps	27	2,4,5	CDS	LN	10	11	3	9	15	19.82	0.42;37.17	18.21	0.4;32.67‘
82	Reps	27	4,5	CDS	LN	10	6	4	5	8	19.85	0.63;38.58	16.60	0.55;34.06
83	Reps	27	5	CDS	LN	10	3	4	3	3	46.51	8.51;26.31	43.83	6.45;23.78
84	Reps	27	1,2,4,5	CDS	N(1,2,4),LN(5)	10	88	26	**947**	**3650**	30.55	17.27;42.99	26.25	12.73;36.39
85	Reps	27	5,G	CDS	LN	10	29	33	9	28	10.23	7.56;13.74	7.92	6.21;9.73
86	All	32	5	CDS	N	100	**5916**	**2594**	**16,477**	**1525**	22.33	15.3;25.14	20.46	13.65;22.53
87	All	32	5	CDS	LN	100	**1256**	**1949**	**1775**	**850**	24.05	17.63;28.31	21.79	15.81;25.44
88	Reps	27	1,4,5	CDS	E	100	**1028**	**362**	**10,408**	**45,050**	21.44	17.93;35.88	19.12	14.59;31.59
89	Reps	27	1,2,4,5	CDS	E	100	**1224**	**343**	**9333**	**35,300**	24.05	17.66;28.31	21.79	15.81;25.44
90	Reps	27	1,2,4,5	CDS	LN	100	**564**	**232**	**8176**	**17,530**	22.33	15.29;25.14	20.46	13.65;22.53

(*continued on next page*)

Table 3 (*continued*)

BEAST2 Run	NZ Species	Number of taxa included	Calibration	Alignment type	Prior distribution	Number of generations	Posterior	Prior	Tree Likelihood	Tree Height	Tas/NZ split age	Tas/NZ 95% HPD	NZ crown group age	NZ 95% HPD
91	Reps	27	1,2,4,6	CDS	N	10	**754**	**439**	**819**	**4285**	32.10	25.398;39.28	27.95	20.49;34.87
92	Reps	27	1,2,4,6	CDS	N	100	**7797**	**4195**	**8574**	**32,463**	32.08	25.30;38.99	27.83	20.75;34.58
93	Reps	27	2,4,6	CDS	N	10	**555**	**212**	**818**	83	31.87	24.19;40.17	27.70	19.42;34.96
94	Reps	27	2,4,6	CDS	N	100	**5958**	**1490**	8684	701	31.90	24.19;39.42	27.63	20.32;34.95
95	Reps	27	4,6	CDS	N	10	163	48	**1141**	47	32.06	24.45;38.90	27.90	20.69;34.01
96	Reps	27	4,6	CDS	N	100	1125	**280**	**8813**	**301**	30.99	23.79;38.56	26.89	19.77;34.20
97	Reps	27	6	CDS	N	10	6	27	5	28	31.50	25.14;38.10	27.31	21.01;33.42
98	Reps	27	6	CDS	N	100	**622**	198	**10,879**	**220**	30.96	22.66;38.40	26.82	18.95;30.06
99	Reps	27	1,2,4,6	CDS	E	10	7	6	7	**546**	31.73	6.77;42.05	27.85	7.60;36.35
100	Reps	27	1,2,4,6	CDS	LN	10	3	3	3	**870**	6.56	0.90;35.55	3.99	0.50;31.23
101	All	32	6	CDS	LN	100	**5506**	**3045**	**15,061**	**1778**	33.04	27.06;40.22	29.53	24.11;36.00
102	All	32	6	CDS	N	100	**5305**	**951**	**15,298**	**1308**	31.46	26.96;35.47	28.09	23.98;21.9

incompatibility of the molecular data with calibration via constraint of the ingroup to the age of the mountains (~5Mya), effectively rejecting our null hypothesis.

Our molecular clock analyses estimate the last common ancestor of the New Zealand alpine grasshoppers was alive in the early–mid Miocene. Palaeoclimate models informed by ocean proxies (Kennett, 1986), macrofossils (Pole and Moore, 2011), and palynology (Prebble et al., 2017) converge on the inference that New Zealand's climate in the early–mid Miocene was warm-temperate nearing subtropical, with high rainfall supporting forest dominated vegetation (McGlone et al., 2001; Reichgelt et al., 2015). The global cooling transition initiated in the middle Miocene (~14 Mya) (Holbourn et al., 2014; Zachos et al., 2001) had a well-documented impact in the New Zealand region that included significant extension of the Antarctic ice sheet (Flower and Kennett, 1994; Shackleton, 1975). Nevertheless, a predominantly low topography in New Zealand at that time would have precluded alpine conditions. Palynological data spanning 30 million years of New Zealand strata do not indicate high elevation biomes until the Pliocene, consistent with Southern Alps orogeny at that more recent time (Prebble et al., 2017). Miocene topography and seasonality might have generated habitat variation in the landscape to support grasshoppers but suitable data are limited. Although inferences from early-mid Miocene (23–11 Mya) fossil leaf assemblages suggest moisture deficit at some sites, this was likely linked to the mid Miocene cooling transition (Reichgelt et al., 2019). Similarly, shifts in isotope ratios of kaolinites have been interpreted as indicating development of orographic rain shadow in South Island, but this appears to have been primarily in Pliocene time (Chamberlain and Poage, 2000; Horton et al., 2016).

New Zealand's alpine grasshoppers are today closely associated with the alpine zone and display freeze-tolerance adaptations, as do their Tasmanian cousins. The parsimonious interpretation is that alpine adaptation is a shared ancestral trait. Tasmania's mountainous topography probably existed throughout the Cenozoic (66–0 Mya), with a sufficiently cool climate for an alpine environment in Tasmania developed by the late Oligocene/early Miocene (~23.7 Mya; (Hill, 1988; Macphail et al., 1991)). Therefore, it is plausible that Tasmania's grasshoppers evolved cold tolerance during the Miocene in a newly formed alpine zone and this is compatible with our fossil calibrated molecular clock analyses that found the most recent common ancestor of the New Zealand and Tasmanian grasshoppers at about this time (15–25 Mya). The difficulty is that estimates for the age of the most recent common ancestor of the monophyletic New Zealand grasshopper clade (13–22 Mya) substantially predate formation of the Southern Alps (~5 My), suggesting that the grasshopper radiation began during a geological period when no alpine habitat would have been available in New Zealand (McGlone et al., 2001). Either the ancestor of the New Zealand grasshoppers arrived once and survived and speciated without alpine habitat, requiring subsequent multiple parallel adaptations to the cold (including freeze-tolerance), or alpine adaptation is an ancestral state and multiple arrivals to New Zealand is required to explain the current diversity. A possible source of alpine (or cold adapted) insects might have been Antarctica or the Sub-Antarctic Islands which would have provided habitat for a diverse plant and insect biota prior to ice sheet extension (Convey et al., 2008). It is conceivable that a cold adapted grasshopper could have colonised Antarctica and/or the Sub-Antarctic Islands ~20 Mya, prior to the peak of the Antarctic greening, diversifying there over the next 14 Mya (Feakins et al., 2012; Lewis et al., 2008) before taking advantage of the new alpine habitat in New Zealand via multiple long distance dispersals. The current absence of grasshoppers in Antarctica and the Sub-Antarctic Islands prevents detection of cold-adapted taxa sister to the New Zealand grasshopper lineage, but it is also well known that tip traits can provide deceptive sampling of ancestral traits and thus we should not necessarily assume the ancestor of the New Zealand lineage was cold-adapted (Crisp et al., 2011; Garcia–R et al., 2019; Grandcolas and Trewick, 2016). Insect cold adaptation can evolve rapidly and independently as demonstrated in a

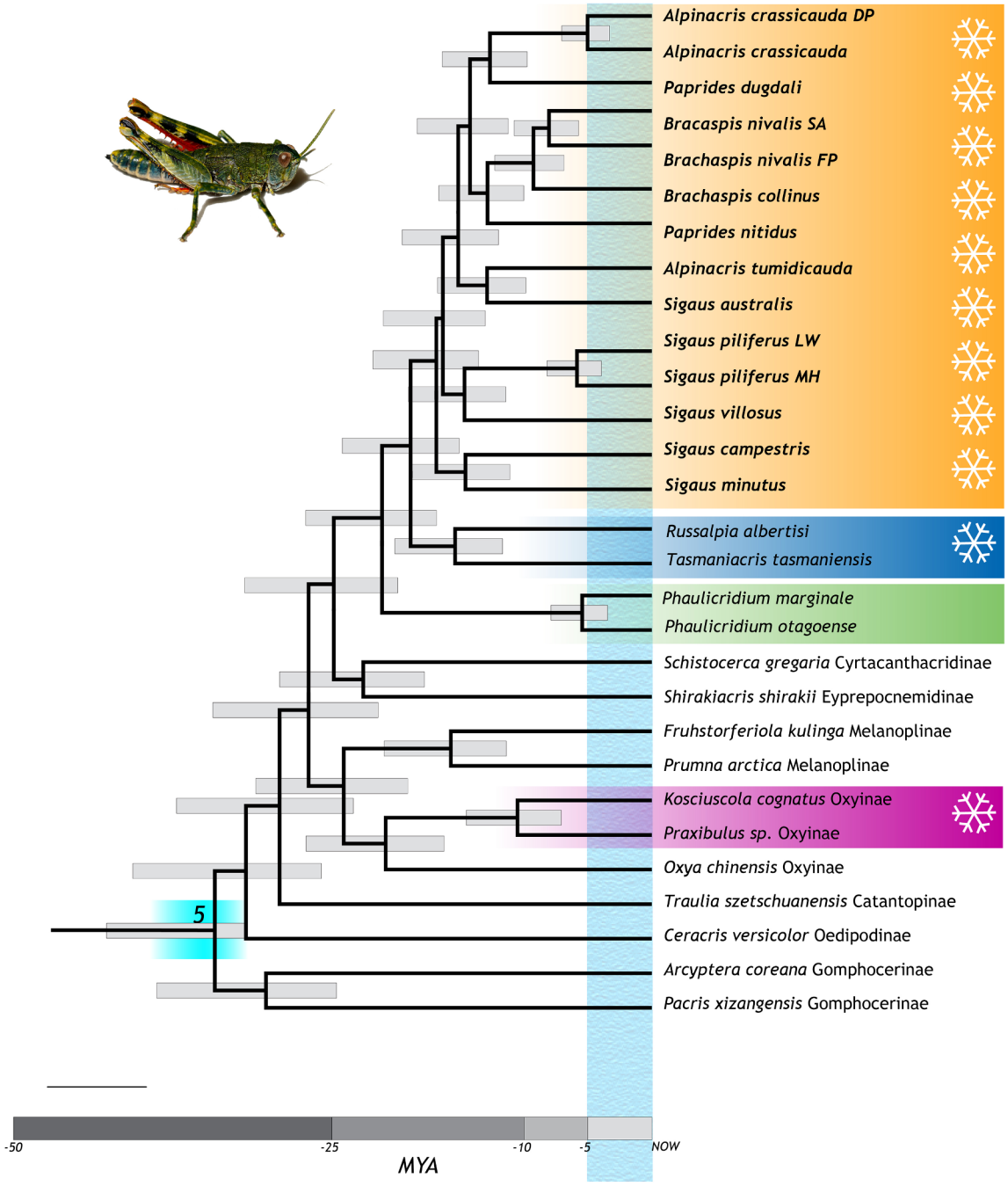

Fig. 5. Phylogenetic hypothesis of Acrididae from fossil calibrated analysis of a 10,015 bp alignment of mitochondrial DNA protein coding sequences from 32 Acridoidea using Bayesian Inference (BI). Time calibration used (turquoise) Acrididae *Tyrbula russelli* (Fossil 5 in Table 2) with an exponential prior distribution at the node leading to the representatives of that family (Run 56 in Table 3) which are presented here. No sequences were enforced as the outgroup. The clade of endemic New Zealand alpine grasshoppers are highlighted in orange, New Zealand endemic lowland grasshoppers in green, alpine grasshoppers from Tasmania in blue and more distantly related alpine grasshoppers from mainland Australia in mauve. Inset: *Brachaspis collinus*, St Arnaud, New Zealand. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lineage of New Zealand phasmids in the same landscape (Dunning et al., 2013).

Aside from the issues of long-distance dispersal (e.g. discussed in Goldberg et al., 2008) the strong contrast between modern and inferred Miocene habitats available in New Zealand appears problematic for grasshopper establishment. As ectotherms requiring solar radiation for thermoregulation, Acrididae are generally abundant in open vegetation (rangeland) habitat that provides opportunity for direct basking (Harris et al., 2015; Pepper and Hastings, 1952). In modern temperate New Zealand diurnal Acrididae are restricted to such open habitats, which differs profoundly from endemic, nocturnal Anostostomatid Orthoptera that are active at night in forests and metabolise at low temperatures (Minards et al., 2014; Trewick and Morgan-Richards, 2019). The ecological diversity of Acrididae reveals their adaptive potential belied by morphological conservatism (García-Navas et al., 2017). Globally, the group is by no means limited to open or dry habitats. Despite the name "grasshopper" they have high diversity in tropical rainforest in the Neotropics (Da Costa et al., 2015; Jago and Rowell, 1981; Rowell, 2013), and many species use low elevation wet habitats including *Stethophyma grossum* on European wet meadows and moss bogs,

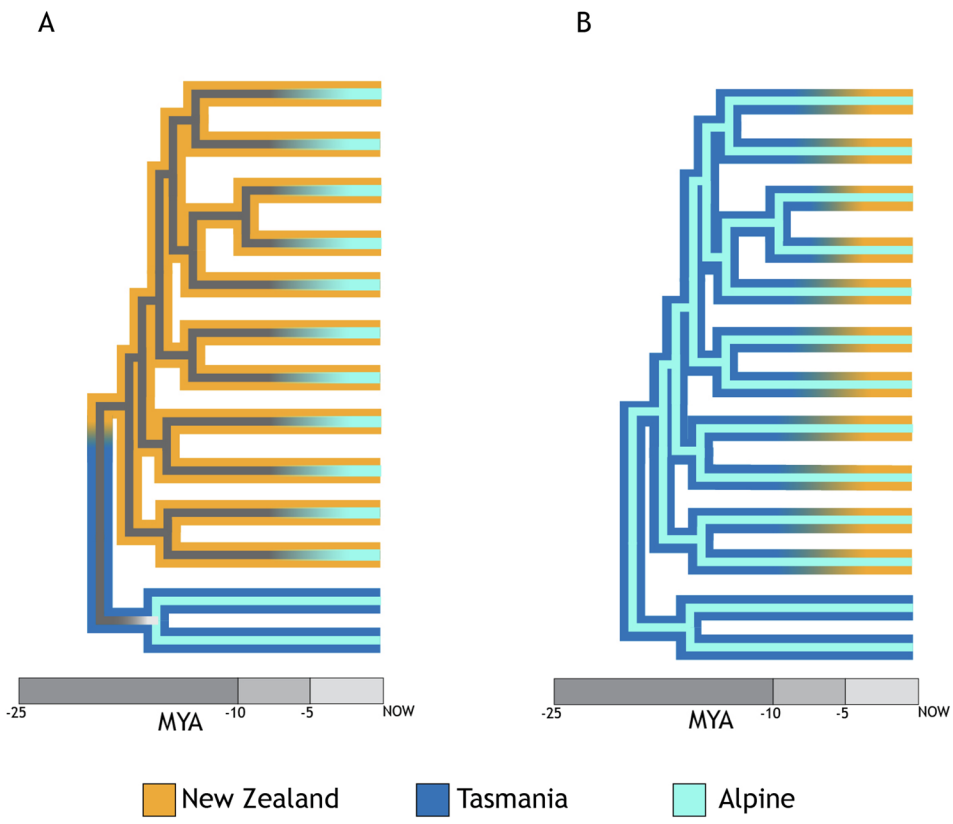

Fig. 6. Alternative hypotheses for colonisation of New Zealand by 'alpine' short-horn grasshoppers. (A) A single founder of the lineage in New Zealand diversifying in available early-mid Miocene warm, wet conditions before occupying montane habitat following Pliocene orogeny, and independent evolution of freeze-tolerance. (B) Multiple colonisations of New Zealand into the Southern Alps by alpine adapted founders. Inner phylogeny depicts ecological trait of grasshopper lineages, while outer phylogeny depicts their geographic trait.

Paroxya clavuliger on swamps and salt marshes in North America and *Paulinia acuminate* in swamps and floating vegetation in the Amazon. Australia has around 3000 grasshopper species that occur across diverse terrestrial habitats in all parts of the country. Oxyinae (including *Kosciuscola* and *Praxibulus* species) live predominantly in wet habitats and display subaquatic behaviour (Rentz, 1996). The ancestors of New Zealand's alpine grasshoppers could therefore plausibly have established themselves in conditions unlike those in which we find them today. Open habitats available for New Zealand grasshoppers are likely to have been the edges of forests, around rivers and lakes, wetlands, swamps, or coastline, wherever local conditions limited forest growth.

One possibility is that the ancestor of the New Zealand high country grasshoppers colonised and diversified without alpine habitat. It is unlikely that alpine physiology was retained for millions of years of low elevation conditions in Miocene New Zealand that would have imposed different metabolic demands. If so, freeze-tolerance adaptations evident today must have subsequently evolved separately in each of several lineages (Fig. 6). Confirmation that the New Zealand/Tasmanian alpine clade is not sister to other Australian alpine species demonstrates independent evolution of alpine adaptation among Australian grasshoppers, and lends some credence to the idea that the trait might be shared-derived in New Zealand. On the other hand, the presence in New Zealand and Australia of closely related taxa in another two lineages of Acrididae (*Locusta*, *Phaulacridium*), two Tettiigonidae (*Caedicia*, *Conocephalus*) and several Gryllidae (*Teleogryllus*, *Bobilla* and others) indicate that multiple over-sea colonisations have occurred in Orthoptera. Numerous arrivals from Tasmania or Antarctica present theoretical challenges that are common in biogeographic interpretation. Their solution requires empirical data about dispersal phenomenon that might involve relatively uncommon, extreme weather events, in order to interpret likelihood. Similarly, empirical data on the mechanisms of habitat switching (cold adaption in this case) would be revealing about the plausibility of convergent evolution among numerous grasshopper lineages. The fact that New Zealand's youthful alpine zone includes many different endemic insects that can survive freezing, including wētā (Ramløv et al., 1992), cockroaches (Block et al., 1998), grasshoppers (Hawes, 2015) and stick insects (Dennis et al., 2015), suggests abundant evolutionary potential exists for exploitation of cold environments (e.g. Bi et al., 2019; Billings, 1974; Sømme and Block, 1991).

Pleistocene climate cycling starting ~2.6 Mya influenced the distribution of New Zealand insect species as in the northern hemisphere (Bulgarella et al., 2014; Morgan-Richards et al., 2010; Trewick et al., 2011). It is likely to have selected for cold adaptation during the glacial phases that extended through the majority of this epoch (Alloway et al., 2007; Trewick and Bland, 2012). Reduction of alpine habitat in New Zealand during the shorter interglacials would likely have emphasised the association of the grasshoppers with high elevation habitat as seen today. However, vegetation communities and grasshoppers can be ecologically decoupled because plant growth is primarily predicted by mean soil temperature during the growing season, whereas insects such as grasshoppers can use direct solar basking to elevate and control body temperature for metabolic activity. This means that on mountains, vegetation height diminishes with elevation and the resulting open environment enables grasshoppers to live despite often low air temperature. Nevertheless, the same or closely related grasshoppers can also survive at lower elevation (e.g. Gillis and Smeigh, 1987) , but only if there are other environmental constraints on forest growth creating open space such as grazing mammals, frost associated with temperature inversion, and fire (Blair et al., 2014; Daubenmire, 1968; Holloway, 1954). This is evident among the New Zealand group of alpine grasshoppers which include species that exist at low elevation. South Island *Sigaus minutus*, *Sigaus childi* (Dowle et al., 2014; Trewick, 2008) and *Brachaspis robustus* (Trewick 2001) live in rainshadow habitat that is known to have been modified by fire (Ausseil et al., 2011). There is also a population of *Alpinacris crassicauda* at about 600 m asl on nutrient-leached, coal measure soils of Denniston Plateau, and in North Island (Te Ika-a-Māui) *Sigaus piliferus* is recorded among harakeke (Asphodelaceae) scrub on rocky outcrops below the climate tree line at several locations (Trewick and Morris, 2008).

4.1. Conclusions

Despite the oft-repeated assertion that current alpine diversity in New Zealand originated after emergence of the Southern Alps, few studies have used independent, taxon-specific calibrations of substantial DNA sequence data to test relevant lineage ages. A review of published data on New Zealand open-habitat plant lineages found that crown ages tended to be inferred as young (Pleistocene) and New Zealand stem ages for many coincided with Pliocene orogeny (Heenan and McGlone, 2013). However, several taxa with origins indicated as Miocene in age may have been associated with open swamp habitat. For animal species, previous analyses have applied generalised rates of mtDNA evolution (e.g. Chinn and Gemmell, 2004; Marshall et al., 2008) or the age of the mountain ranges themselves (e.g. Buckley et al., 2001). Both approaches make many untestable assumptions, and the latter is built on the very idea it seeks to test (Crisp et al., 2011). However, analysis of New Zealand cicadas calibrated by the age of nearby islands bearing congeneric species revealed pre-Pliocene crown age for montane *Maoricicada*, interpreted as evidence of an ancestral use of lowland habitat (Buckley and Simon, 2007). Our fossil calibrated analysis of New Zealand Acrididae indicates diversification a considerable time (~12 My) prior to the orogenic activity that gave rise to the dominant mountain terrain in New Zealand. This finding not only runs counter to inferences about the role of mountain formation in other New Zealand species radiations but suggests multiple independent adaptations to high elevation environments. Convergence of cold-tolerance has been reported in stick insects (Dennis et al., 2015) and freeze tolerance is widespread amongst insects (Sinclair and Chown, 2005), but this alpine grasshopper lineage is monophyletic which explains why it was previously thought to be only as old as the mountains of Aotearoa.

CRediT authorship contribution statement

Emily M. Koot: Conceptualization, Formal analysis, Investigation, Writing - original draft. **Mary Morgan-Richards:** Conceptualization, Supervision, Resources, Writing - review & editing. **Steven A. Trewick:** Conceptualization, Supervision, Resources, Visualization, Writing - review & editing.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2020.106783.

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